Peer Review
Experimental Toxicology Division
NHEERL

PARTICULATE MATTER

Comparative Toxicity of Coarse, Fine, and Ultrafine Particles from Combustion Sources and Ambient Air

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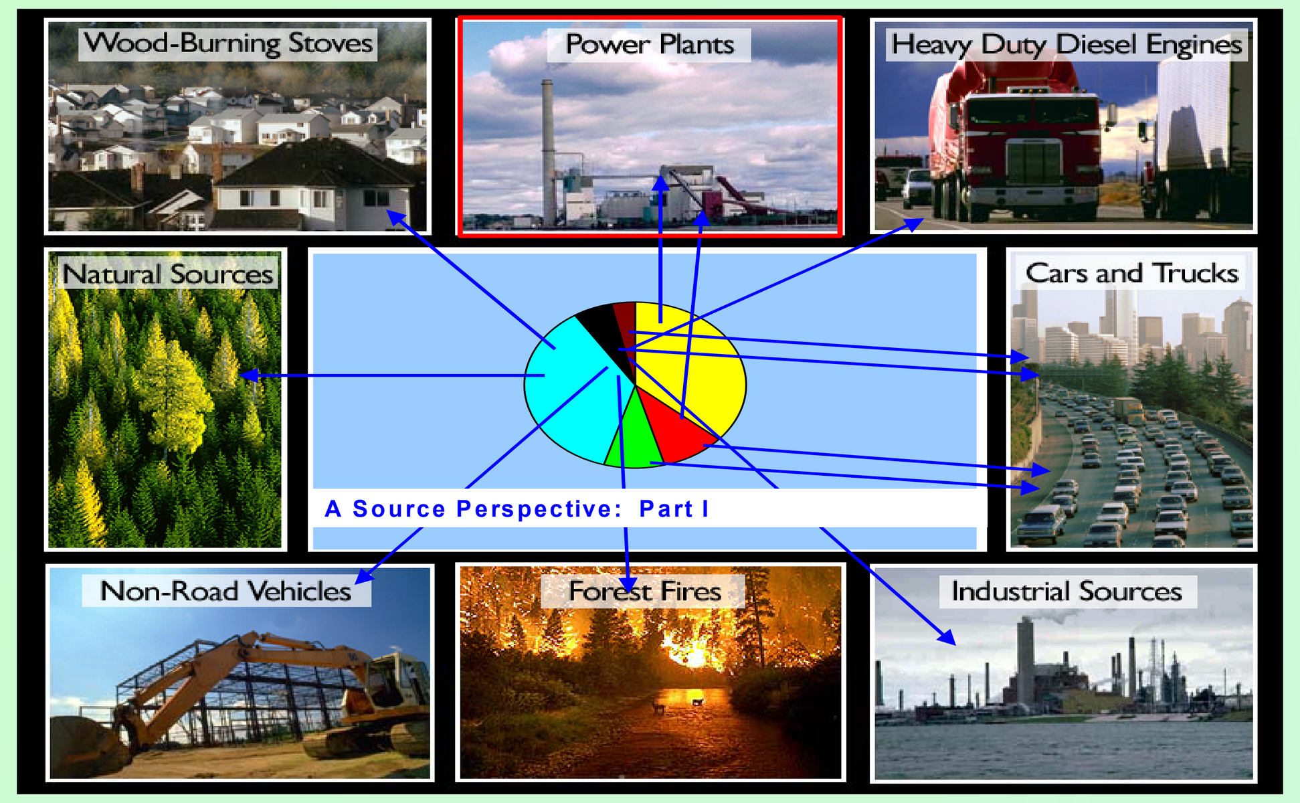
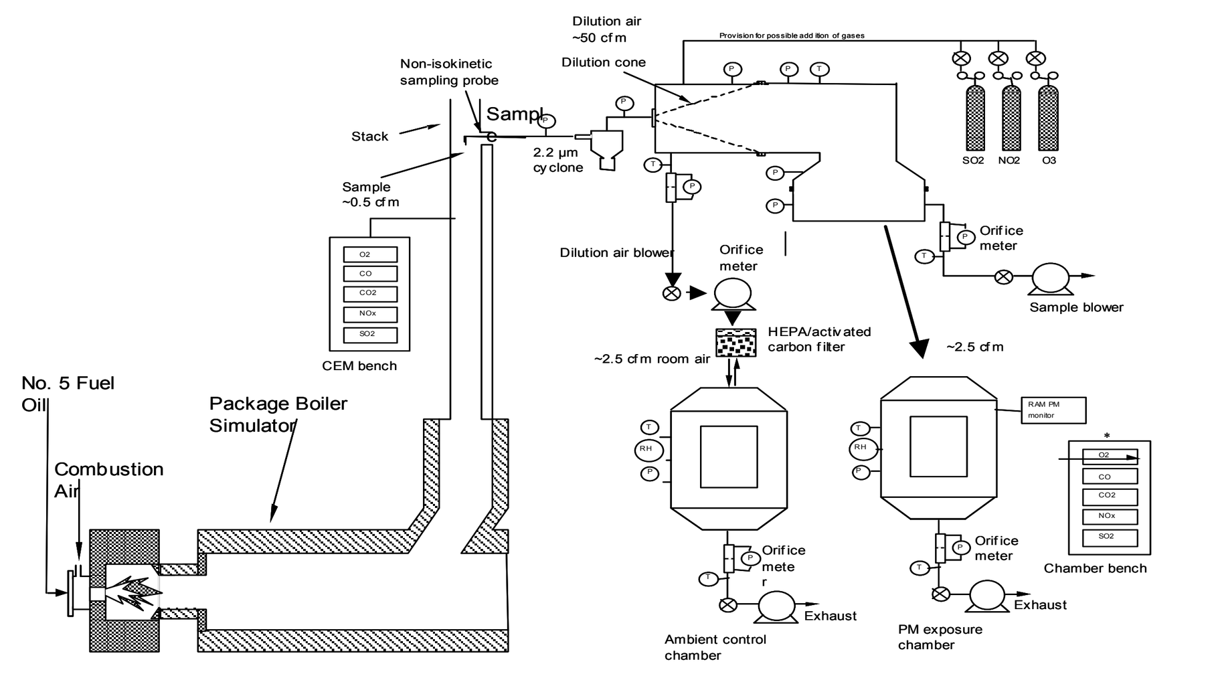
Introduction

Despite overwhelming evidence showing that PM exposure increases morbidity and mortality the relative contribution of different sources and individual components of PM is still not known. The goal of this research program is to assess the toxicity of inhaled or instilled PM of different size fractions from combustion sources and to compare these effects to particles collected from ambient air. To date inhalation studies have been conducted with concentrated air particles and combusted oil emissions while instillation studies have been performed with a number of size fractionated ambient PM samples, coal fly ash and different types of diesel.



High Volume Dilution Sampler/Whole Animal Exposure Facility



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Methods and Approach

For the inhalation studies rats and mice were exposed for 4-6 hours to concentrations of emission aerosol up to 3 mg/m-3. Animals were necropsied immediately and 20 hr post exposure and local and systemic markers of injury and inflammation were assessed in the lungs and blood at various times afterwards. In the mouse bioassay system animals were instilled with doses of various PM up to 100 µg and pulmonary endpoints of lung injury and inflammation assessed at various times post-instillation. In addition to outbred CD1 mice, endotoxin resistant and sensitive C3H mice were used to determine the role of Tlr 4 receptors on pulmonary inflammation, and whether endotoxin plays a role in health effects of ambient PM.

Oil Combustion Inhalation

Combustion Emission Aerosol System (CEAS) Operating Conditions

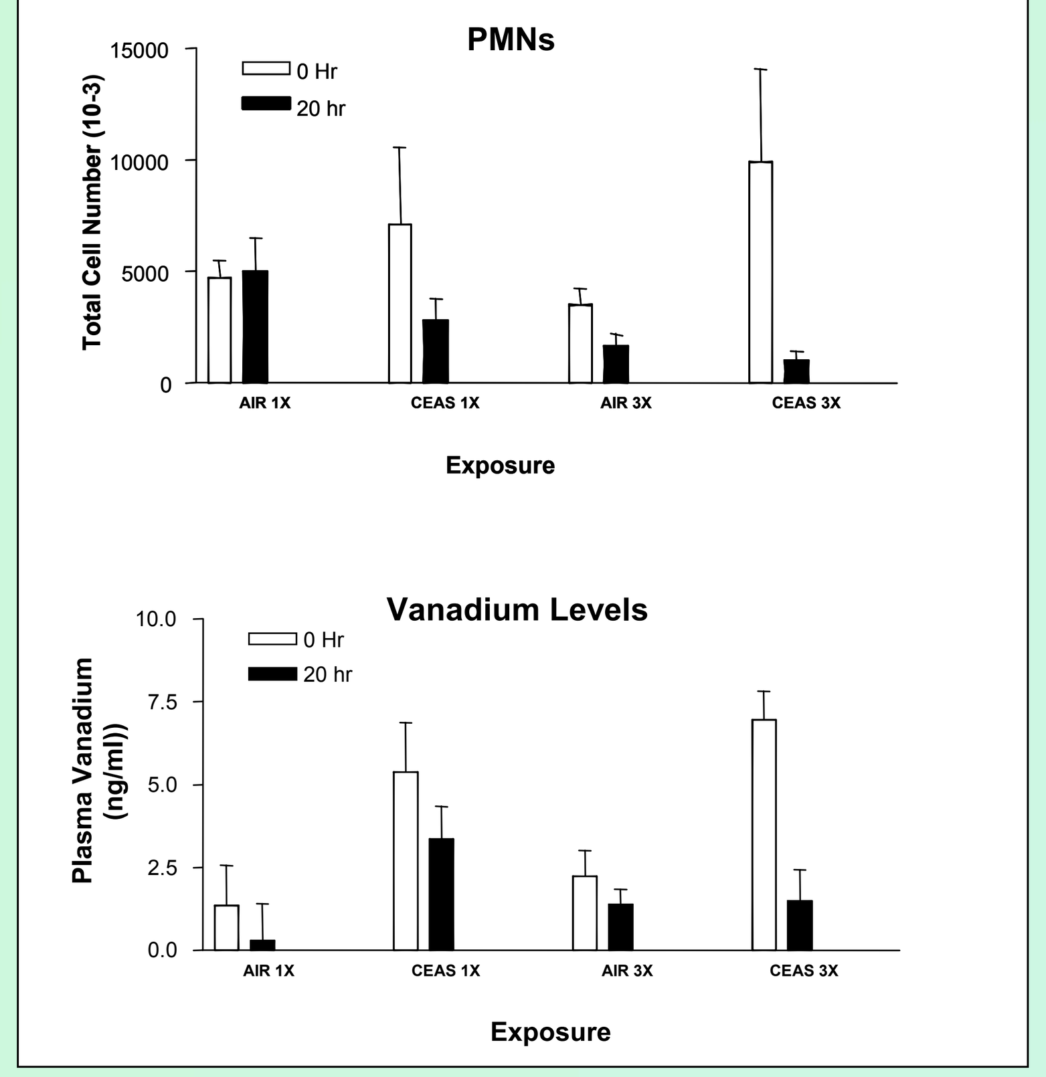
Stack

Firing rate (°K)	PM mass (mg/m^{-3})	O_2 (%)	CO2 (%)	CO (ppm)	Nox (ppm)	SO2 (ppm)	Moisture (%)
286	86	3.3	13.7	21	340	1204	9.2
241	83	3.9	12.8	22	355	1165	9.5
271	83	3.3	13.9	38	284	1204	9.7

Chamber

Exposure	PM mass (mg/m^{-3})	O_2 (%)	NOx (ppm)	SO2 (ppm) Pre-denude	SO2 (ppm) Post Denude	Temp (°F)	RH (%)
1x	3.8	19.9	21	42	1	71.7	9.2
2x	2.7	19.9	21.5	41	0	71.3	9.5
3x	2.5	19.7	22.2	45	0	72.9	9.7

Effect of exposure to 2.5 mg/m^{-3} combusted oil emission (CEAS) on BAL PMNs and Vanadium levels in plasma.



Exposure to a combustion emission aerosol (CEAS) resulted in an immediate increase in neutrophils which was associated with increased levels of vanadium in the plasma. These effects returned to control (air exposed levels) by 20 hrs post-exposure. 1X = 1 day, 3X = 3 days.

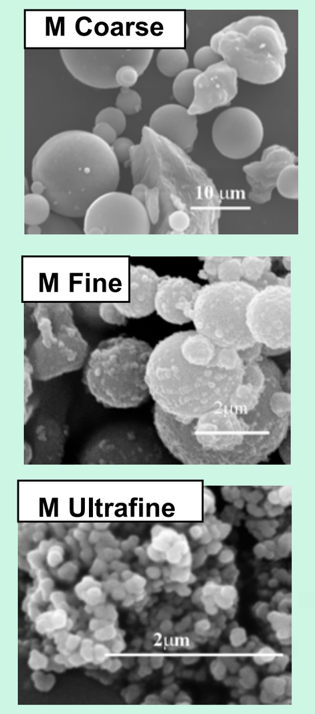
Coal Fly Ash Instillation

Elemental analysis of ultrafine, fine and coarse coal fly ash

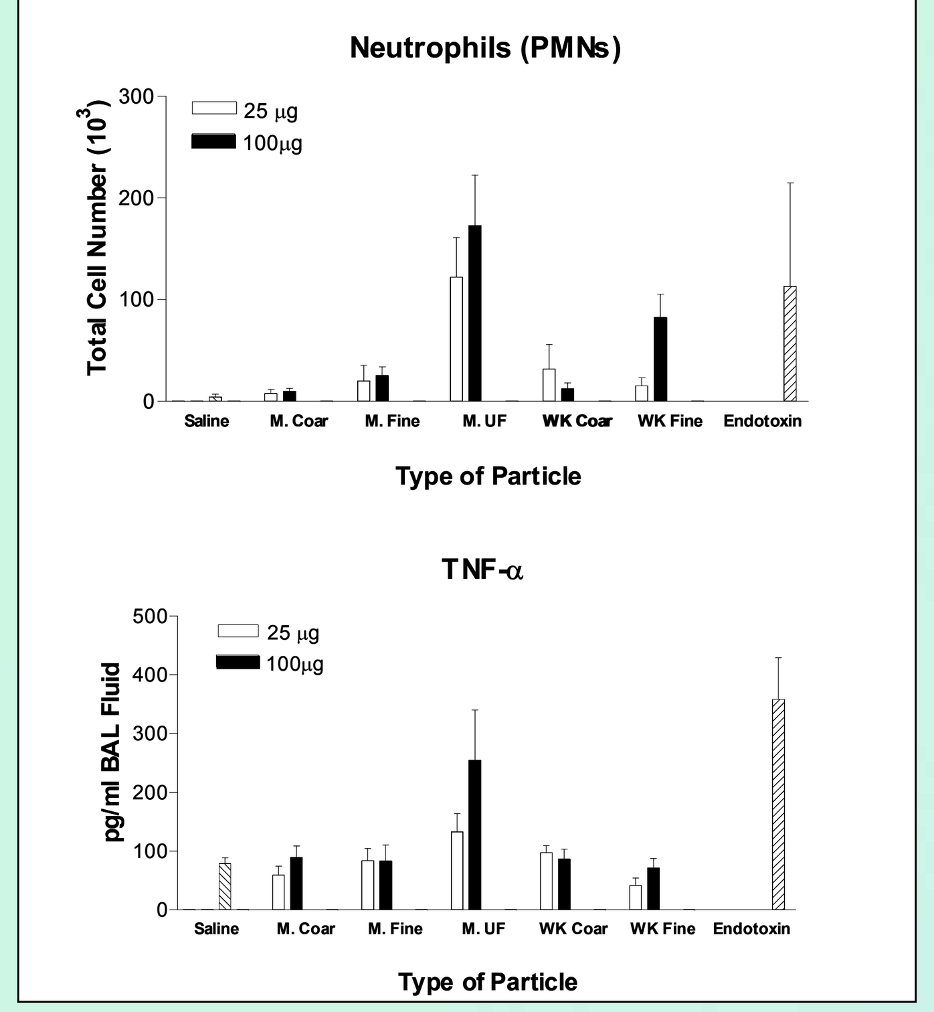
Element µg/g ash	MT UF	MT <2.5µm	MT>2.5µm
Si	28,500	156,742	222,875
Al	93,780	103,979	108,800
Ca	82,900	89,858	115,175
Fe	6,920	53,929	30,350
S	39,400	7070	9,130
Mg	14,600	27,721	31,300
Ti	1845	6353	6180
K	1155	9358	5660
Cl	659	1264	1460
Ba	16200	2298	1843
P	10530	1080	979
Sr	7480	3426	3858
V	712	208	108
Ni	330	347	
Nb	910	176	22
Mn	487	1048	907
Cd	1620	463	
Se	565	136	
Ga	460	83	27
Cu	420	320	77
Elements %	22.5	47	54
Oxygen %	16.5	44.5	45
Carbon %	unknown	0.4	0.5

Ultrafine enriched

Coarse/Fine enriched



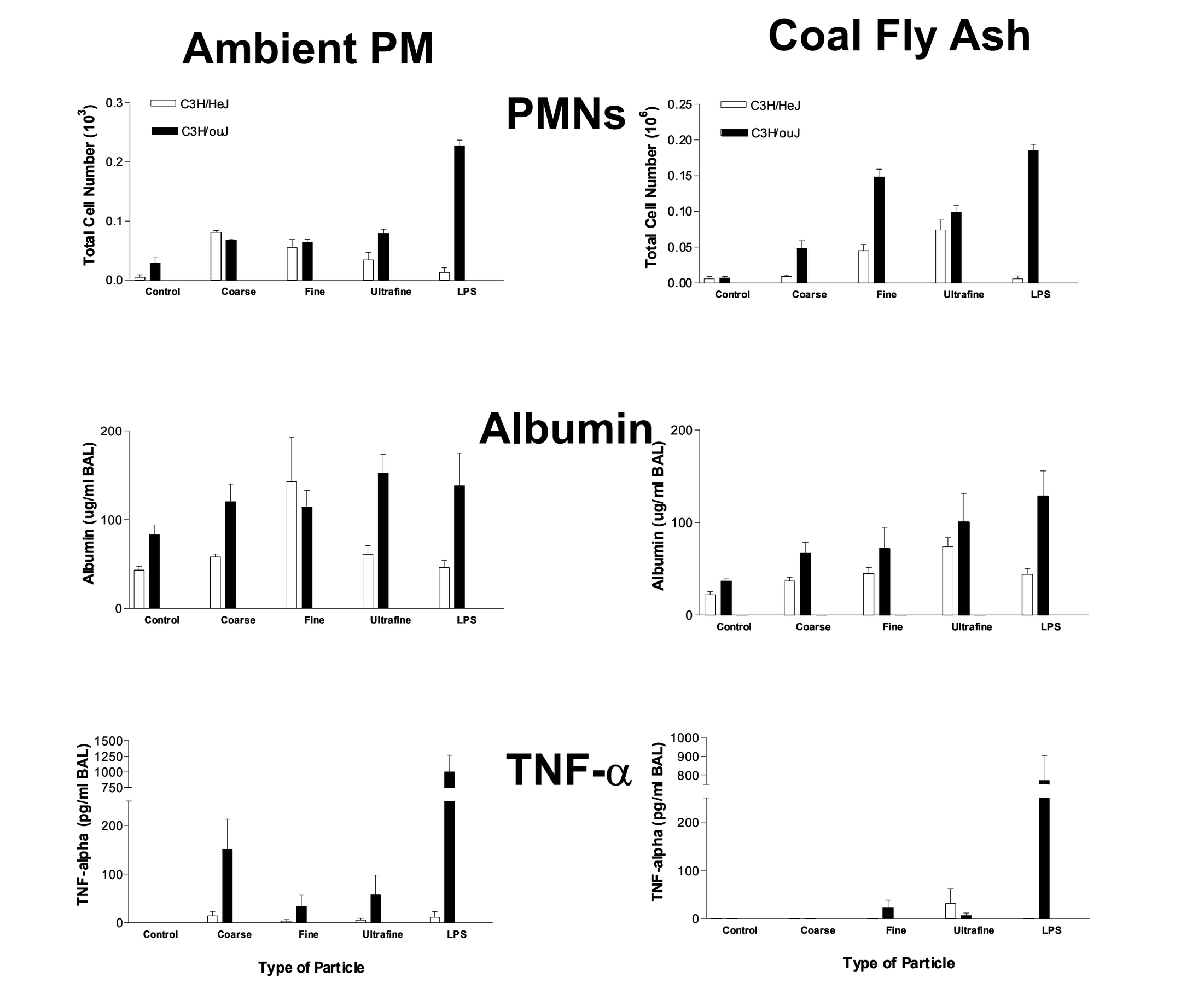
Effect of Coarse, Fine and Ultrafine Coal Fly Ash Instillation on Inflammation in Mouse Lungs.



An inverse relationship exists between coal fly ash particles sulfur content, and toxic trace elements. Ultrafine fly ash particles are more inflammogenic than fine and coarse particles from the same coal source (Montana), and W. Kentucky fine particles are more toxic than Montana fine particles.

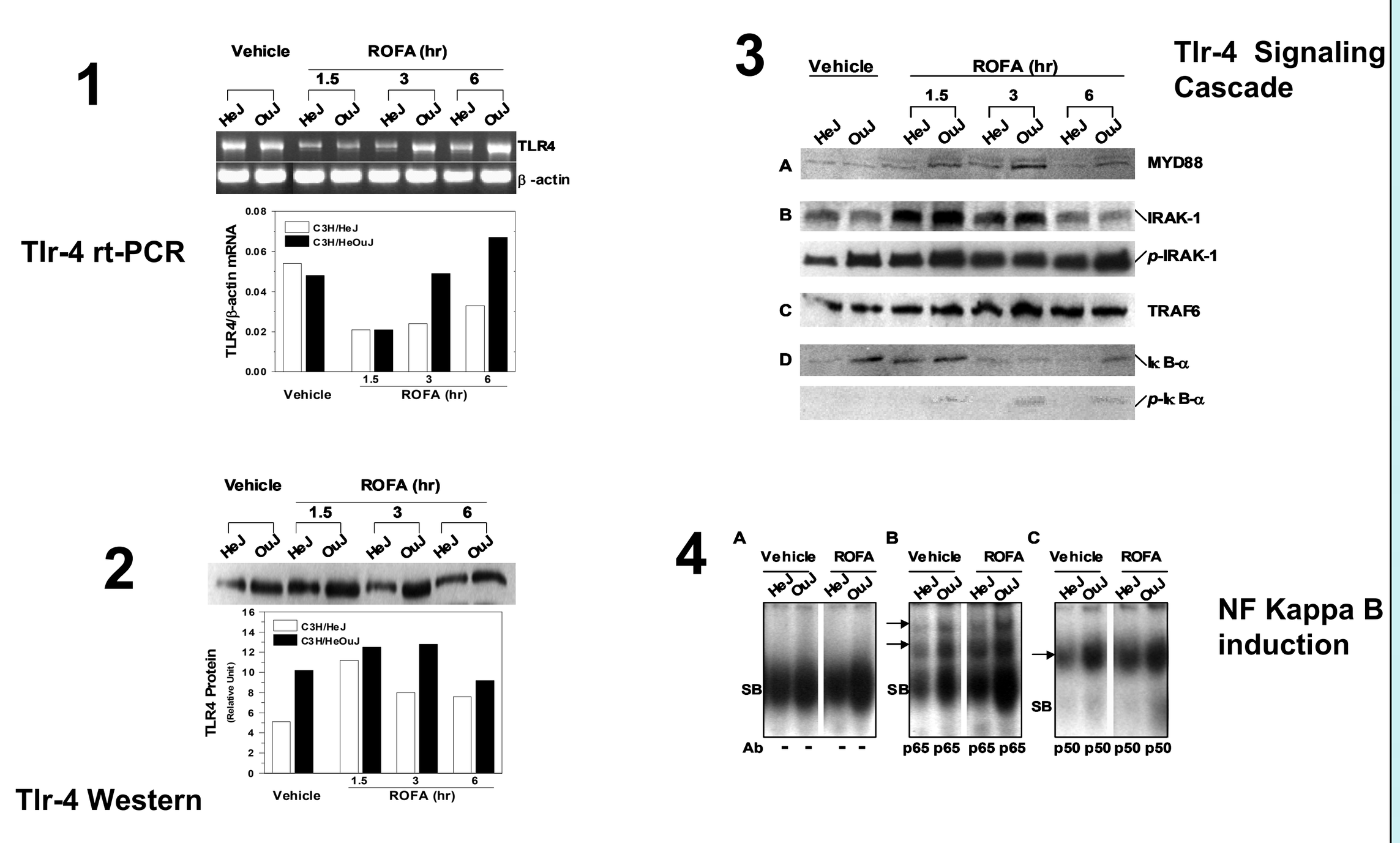
Effect of Ambient and Combustion PM on Pulmonary Inflammation in Tlr-4 Sufficient and Deficient Mice

Effect of Instillation of Size Fractionated Ambient PM or Coal Fly Ash on Pulmonary Inflammatory Resposes in Endotoxin Resistant (HeJ) and Sensitive (Ouj) Mice



Ambient PM causes increased cytokine release and albumin levels in endotoxin sensitive mice suggesting a requirement for Tlr-4 functionality. No differences in pulmonary neutrophils were evident across the various size fractions suggesting that LPS was not required for the this effect and that Tlr-4 receptor signaling is not involved.. Coal fly ash causes lower amount of cytokine and albumin levels but increased PMNs in the coarse and fine modes of OuJ mice suggesting a Tlr-4 requirement.

Effect of Combustion PM Exposure on Tlr-4 Signaling Cascades



Tl4-4 m RNA expression (1), protein levels (2) as well as signaling molecules (3) and NF Kappa B induction (4) are higher in endotoxin sensitive mice (ouj) exposed to ROFA compared to endotoxin resistant mice (HeJ).

Conclusions

The combustion emission experiments show only modest and transient effects on pulmonary inflammation in outbred rats at concentrations between 2 and 3 mg/m-3 despite increased metals in the plasma.

Instillation of coal fly ash in outbred mice caused a dose dependent increase in pulmonary inflammatory responses with ultrafine particles being more potent on a mass basis which may reflect increased amounts of metal compounds.

Instillation of size fractionated ambient PM and coal fly ash particles in endotoxin resistant and sensitive mice showed different patterns or inflammatory responses which are associated with their differing origin and chemistry.

Differential responses between size and type of PM in endotoxin sensitive mice show a partial dependence on Tlr-4 signaling

Expression and production of Tlr-4 and its associated signaling molecules is stronger in endotoxin sensitive mice exposed to ROFA

Impact

The development of combustion exposure systems using oil, coal and diesel fuel will enable us to test the relative toxicity of PM from different sources and size fractions, and identify chemical components which cause these effects. Combustion conditions may also be altered to provide different aerosol characteristics and chemistry. While inhalation exposures are the preferred route of exposure, collection of material for instillation and *in vitro* studies can provide fast comparative data across species and strains. The use of Tlr-4 sufficient and deficient animals provides information on mechanisms of inflammation and toxicity, and whether LPS is the only active component in ambient air samples. Finally, examination of signaling cascades and transcription factors in parallel with component analysis may allow identification of sources in ambient PM samples through common biological effects mechanisms.

Future Directions

The exposure system is being expanded to test size fractionated products of coal and diesel combustion under a number of operating conditions. Instillation and in vitro studies are underway to provide relative toxicity data of various source PM materials and to determine species differences between human and rodent pulmonary macrophages and epithelial cells.

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